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A SPECIAL "SHEEP DISEASES" NUMBER.

THE popularity of the "Special Number" series of THE VETERINARY JOURNAL has been manifested by the congratulatory messages which have been received from our correspondents and readers, not only from Great Britain and the Dominions, but also from our colleagues in America and on the Continent. Amongst other subjects, Special Numbers have already appeared on Surgery, Physiology, Tuberculosis, Canine Medicine, and the Diseases of Cattle and Poultry, and the April issue will be entirely devoted to the Diseases of Sheep; whilst at a later date in the year those of some of the other farm animals, especially the pig, will be dealt with. The sheep has been one of the more fortunate of the farm animals in respect to the successes attained during the last decade in the efforts to control its epidemic disease, but much yet remains to be done, and as many of the diseases of these animals are seasonal, the progress must necessarily be slow. The want of pecuniary support for veterinary research, both from the side of the Government and that of the agricultural community, as well as from the general public—all of whom are directly interested in the eradication of animal epidemics from the country—has been felt for many years; and in comparison with what is done for research into animal diseases in other countries the situation would be ludicrous were it not so serious.

It is to amend this state of affairs that the Agricultural Research Council has come into being, and there is a prospect that veterinary research may at last have an opportunity to come into its own.

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General Articles

PROTECTION OF HORSES AGAINST TETANUS BY ACTIVE IMMUNISATION WITH ALUM TOXOID.

By A. T. GLENNY, ANNIE G. HAMP, and MURIEL F. STEVENS.
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HORSES do not possess any natural immunity to tetanus. Buxton and Glenny (1921) tested 500 horses, and found no normal tetanus antitoxin. This, together with the susceptibility of horses to tetanus infection and the ever-present risk of infection, necessitates the adoption of prophylactic measures. In this country the injection of tetanus antitoxin is the usual method adopted, *i.e.*, the animals are passively immunised, and so protected for a short space of time only. It is possible, however, to practise methods of active immunisation which render the treated animals immune to tetanus infection for long periods.

Active immunisation may be brought about by means of toxin rendered harmless by the addition of antitoxin. Buxton and Glenny (1921) showed the practicability of the application of this method with regard to tetanus. Tetanus toxin acted upon by certain chemicals has also been used in the active immunisation of animals. Vallée and Bazy (1917) used Gram's iodine to render toxin non-toxic, and protected horses by its use. Within recent years formol toxoid (toxin rendered harmless by the addition of formaldehyde) has come greatly into prominence, especially in connection with diphtheria immunisation. Toxoid was the term originally given by Ehrlich to that modification of toxin which is no longer toxic, but is still capable of uniting with anti-toxin ; in other words, the toxin is harmless, but is still antigenic, being capable of stimulating the production of antitoxin in an injected animal. Lowenstein (1909) modified both diphtheria and tetanus toxins by this method, though the results were not completely satisfactory. Glenny, in 1904, used formol toxoid in connection with diphtheria immunisation, and Glenny and Sudmersen (1921) were the first to immunise guinea-pigs successfully by means of diphtheria toxin rendered non-toxic by the action of formaldehyde. Glenny and Hopkins (1923) suggested the use of this preparation for human immunisation, and Descombey (1925), working with tetanus, recommended the use of formol toxoid in the active immunisation of man and horses. Ramon (1924) applied the term " anatoxine " to this modification.

Glenny and Pope (1925) suggested that the rate at which an antigen is eliminated after injection governs, to a large extent, the resultant degree of immunity. Ramon (1925) and Ramon and Descombey (1925) added tapioca to diphtheria and tetanus anatoxines used in immunising horses, and obtained better immunity ; they suggested that the local reaction produced by such a mixture prevented

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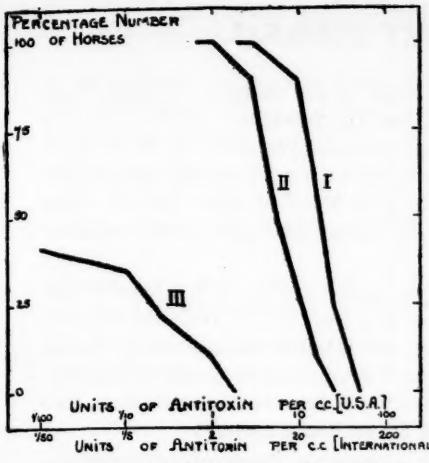

CHART I.—Showing the percentage number of horses with different degrees of immunity response, 3–4 weeks after the injection of tetanus toxoid A, with and without the addition of 1 per cent. alum.

Curve I.—A+1 per cent. alum—50 c.c. doses—4th week value—8 horses.
Curve II—A+1 per cent. alum—10 c.c. doses—3rd week value—10 horses.
Curve III.—A without alum—50 c.c. doses—4th week value—9 horses.

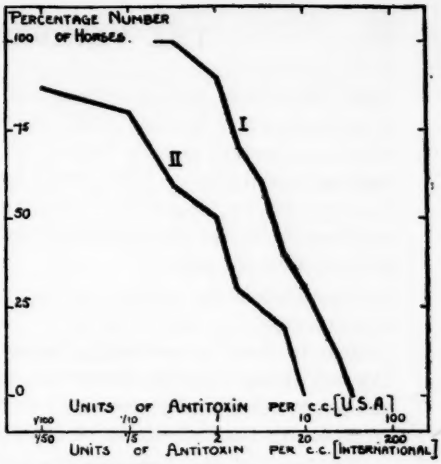

CHART 2.—Showing the percentage number of horses with different degrees of immunity response, 3–4 weeks after the injection of tetanus toxoid B, with and without the addition of 1 per cent. alum.

Curve I.—B+1 per cent alum—50 c.c. doses—4th week value—10 horses.
Curve II.—B+1 per cent. alum—10 c.c. doses—3rd week value—10 horses.
B without alum—50 c.c. doses—after 4 weeks all 9 horses had no detectable antitoxin.

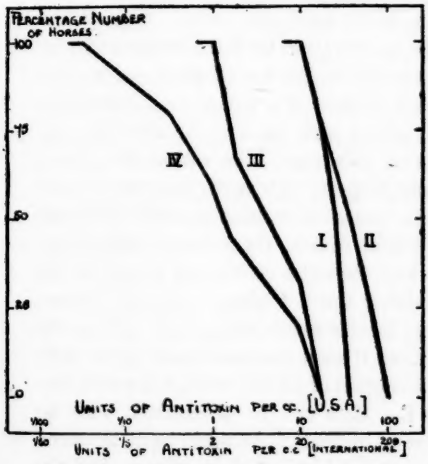

CHART 3.—Showing the percentage number of horses with different degrees of immunity response as the result of a secondary stimulus of tetanus toxoid, with and without the addition of 1 per cent. alum.

Toxoid A (Dose 50 c.c.—interval 4 weeks.)

Curve I:	Primary Stimulus	+Alum	4 horses
	Secondary Stimulus	+Alum	
Curve II:	Primary Stimulus	+Alum	4 horses
	Secondary Stimulus	—Alum	
Curve III:	Primary Stimulus	—Alum	3 horses
	Secondary Stimulus	+Alum	
Curve IV:	Primary Stimulus	—Alum	5 horses
	Secondary Stimulus	—Alum	

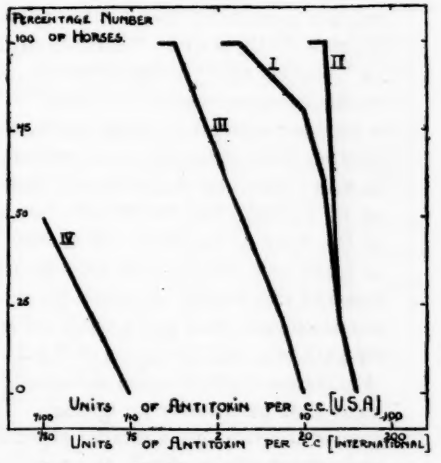

CHART 4.—Showing the percentage number of horses with different degrees of immunity response as the result of a secondary stimulus of tetanus toxoid, with and without the addition of 1 per cent. alum.

Toxoid B (dose 50 c.c.—interval 4 weeks).

Curve I:	Primary Stimulus	+Alum	5 horses
	Secondary Stimulus	+Alum	
Curve II:	Primary Stimulus	+Alum	5 horses
	Secondary Stimulus	—Alum	
Curve III:	Primary Stimulus	—Alum	5 horses
	Secondary Stimulus	+Alum	
Curve IV:	Primary Stimulus	—Alum	4 horses
	Secondary Stimulus	—Alum	

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rapid elimination and consequent loss of much of the antigen. Glenny, Pope, Waddington, and Wallace (1926) showed that the precipitate produced by the addition of potash alum to toxoid had high antigenic properties far in excess of those of toxoid alone. Alum toxoid appears to be the most effective preparation yet reported for the protective immunisation of horses against tetanus. Some evidence of this has already been published by Glenny (1930), and further evidence is detailed in this paper.

To ascertain the efficiency of any prophylactic measure, it is necessary that some method of testing be devised. The antigen itself can be tested for antigenic content to show its immunising properties, and injected animals can be further injected with unaltered toxin, the dose failing to cause death or symptoms indicates the degree of immunity produced ; or the serum of test animals which have been treated with the antigen can be examined for antitoxin content.

(A) *To Test the Antigenic Content or Combining Power of an Antigen.*— The immunising value of any material depends on the amount of specific antigen present. Such added materials as tapioca, alum, etc., may greatly increase the immunising value, but the specific antigen must be present in a sufficient amount. The quantity of the antigen present in any given material may be measured by the amount of antitoxin necessary to neutralise it. Toxins and toxoids are equally antigenic, and as toxins invariably contain some toxoid, tests carried out to ascertain the amount of toxin present, *i.e.*, the M.L.D. test, will not necessarily give a true indication of its antigenic value. Glenny and Walpole (1915) state : " A batch of toxin is appraised by the immunologist by its " binding unit " content per c.c., a quantity which for all purposes is most readily measured as its L+ dose." (The L+ dose of a toxin, *e.g.*, diphtheria, is the least amount of toxin which, when mixed with one unit of antitoxin and injected subcutaneously into guinea-pigs of 250 gms., kills within five days.) Ramon (1924) also states that the antigenic property of a toxin does not depend on its toxicity, *i.e.*, its M.L.D., but on its power of combining with antitoxin at the time of injection. In freshly prepared toxins, the relative proportions of toxin to toxoid do not vary greatly, and thus the combining power of the material can readily be tested by determining the L+ dose. For this reason, immunologists were not greatly misled in former years when they judged the strength of a toxin in terms of M.L.D. Even if such a measurement gives sufficiently close approximation of the antigenic content of a fresh toxin, it is a measurement that need never be applied. The L+ dose can be determined with far greater accuracy and a smaller expenditure of animals. As toxin ages, however, or is chemically modified, the percentage of toxoid to toxin increases, and the M.L.D. test becomes utterly misleading. As further modification takes place, it is no longer possible to use the L+ dose. Ramon (1924) introduced into diphtheria work the flocculation test, the best means at present available for measuring what is recognised as combining power, and has displaced more cumbrous methods, but, unfortunately, with the tetanus toxin at the authors' disposal, the flocculation test frequently fails.

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A method of determining the value of tetanus toxoid has been elaborated and is as follows. The L+ dose of the toxin when freshly prepared is determined by titration in mice against 0.2 International unit of antitoxin. After treatment with formaldehyde, a " total combining power " test is made by mixing varying amounts of the toxoid with 0.2 International unit of antitoxin ; the mixtures are left for one hour before the addition of half a test dose of standard toxin. After a further half-hour, injections are made intramuscularly into mice. The amount of toxoid in the mixture which kills a mouse within ninety-six hours is taken as an index of combining power. (This amount is not exactly equivalent

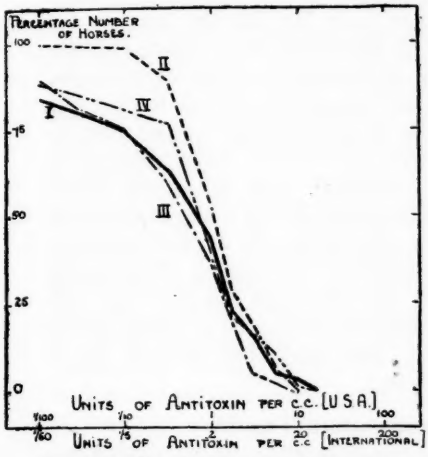

CHART 5.—Showing the percentage number of horses with different degrees of immunity response as the result of a *primary* stimulus of tetanus alum toxoid, injected as whole material, or as a suspension in saline.

Curve I.—C+0.5 per cent. alum—10 c.c. doses—4th week value—65 horses.
Curve II.—C+1 per cent. alum—10 c.c. doses—4th week value—51 horses.
Curve III.—C+1 per cent. alum suspension—10 c.c. doses—4th week value—53 horses.
Curve IV.—C+2 per cent. alum suspension—10 c.c. doses—4th week value—48 horses.

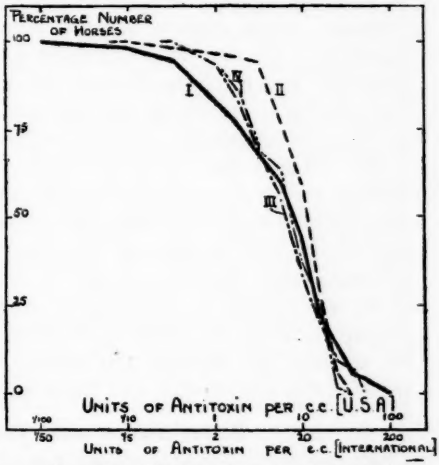

CHART 6.—Showing the percentage number of horses with different degrees of immunity response as the result of a *secondary* stimulus of tetanus alum toxoid, injected as whole material or as a suspension in saline.

Curve I.—C+0.5 per cent. alum—10 c.c. doses—4th week value—62 horses.
Curve II.—C+1 per cent. alum—10 c.c. doses—4th week value—50 horses.
Curve III.—C+1 per cent. alum suspension—10 c.c. doses 4th week value—51 horses.
Curve IV.—C+2.0 per cent. alum suspension—10 c.c. doses—4th week value—46 horses.

to half a test dose, because added toxin used to determine the amount of antitoxin uncombined with the toxoid, and so available for combination with the added toxin, not only combines with the free antitoxin but displaces an unknown amount of the toxoid from combination with the antitoxin.)

(B) *To Test the Immunising Value of an Antigen.*—Laboratory animals, *e.g.*, guinea-pigs, mice, etc., may be injected with the antigen, and, after a suitable interval, may be injected with suitable doses of unaltered toxin, or they may be bled, and their serum tested for antitoxin content. In the former method only one test can be carried out, because if the test animal survives the test dose a larger dose cannot be given after another interval, as the first dose will have

acted as a secondary stimulus. In this connection, Glenny and Sudmersen (1921) working with diphtheria and other toxins showed that the injection of an antigen into a normal susceptible animal is followed by a latent period of several weeks before antitoxin can be detected in the blood: this small and gradual response to an initial injection is termed a primary stimulus response. If, however, a basal immunity existed, *i.e.*, the animal had already received an injection of antigen on any previous occasion, any subsequent injection of antigen is followed by a latent period of a few days only, and the amount of antitoxin produced is 10 or 100 times that produced by a stimulus of the same magnitude in a normal animal; this more rapid response is termed a secondary stimulus response. These authors showed, moreover, that the interval between the injection of the two doses of antigen greatly influenced the secondary stimulus response; thus if the two doses are given at an interval of at least several weeks, the response is much more efficient than if the interval is only one week. This observation is made use of in the production of tetanus hyper-immune serum in horses. Horses that have previously received protective inoculation against tetanus are far more efficient producers of antitoxin upon subsequent hyper-immunisation than are horses that receive an uninterrupted course of injections without an interval elapsing to allow the establishing of a basal immunity (Glenny, Pope, Waddington and Wallace (1925)). The testing of the serum of injected animals is carried out by mixing fixed doses of a standard toxin with varying doses of the serum to be tested, and after contact for a varying period the mixtures are injected into animals, *e.g.*, mice, guinea-pigs, etc., by one or other of the usual routes, and the results noted.

Experiments with Tetanus Alum Toxoid.

Glenny (1930) recorded results of the immunisation of guinea-pigs with tetanus alum toxoid, and showed that all four guinea-pigs injected with 5.0 c.c. alum toxoid tolerated, one month later, 100 test doses of toxin (about 20,000 M.L.D.s) while of thirteen injected with the same volume of the same toxoid without the addition of alum, only three survived 1/50 test dose. In all later experiments, including those recorded in this paper, the immunity produced was measured by the antitoxic content of the serum of the immunised animals.

In Table 1 are given the results of a comparison between the antigenic efficiency of two toxoids of different combining power. The table records the immunity response to the subcutaneous injection of different doses of the toxoids with and without the addition of 1 per cent. alum. Eight guinea-pigs were injected once with each dose, and six weeks later 5 c.c. of blood were withdrawn from the heart of each. The serum obtained from each group of eight guinea-pigs was pooled, and divided into two lots for testing purposes. This permitted a number of tests to be made. In most instances there is a close agreement between both lots of serum pooled from animals of the same group. It will be noted that the combining power of toxoid A was two and a-half times that of toxoid B, and that the doses necessary to produce similar degrees of immunity as indicated by

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the amounts of antitoxin per c.c. in the injected guinea-pigs have the same ratio. The result of this experiment is that guinea-pigs injected with 0.1 c.c. of two toxoids to which 1 per cent. alum was added, one two and a-half times stronger than the other, are as highly immune as guinea-pigs injected with 5.0 c.c. of the same toxoids to which no alum was added. (Table 1.)

In the experiment the results of which are recorded in Table 2, toxoid C, with a combining power intermediate in strength between A and B, was used. The experiment included a comparison between the effects of adding 0.5 and 1.0 per cent. alum to the toxoids, and the injection of the whole mixture (toxoid + alum), and a saline suspension of the washed precipitate. As little of the antigen was precipitated by 0.5 per cent. alum, the separated precipitates formed by 1.0 and 2.0 per cent. alum only were tested. The results of the experiment, though somewhat irregular because of the great variation among guinea-pigs, showed that the antigenic efficiency in descending order was : 2 per cent. suspension, 1 per cent. whole material, 1 per cent. suspension, and 0.5 per cent. whole material. (Table 2.)

Experiments in Horses.—Individual variation of animals in response to the injection of an antigen depends largely on condition. The horses on which our observations have been made in connection with this work are those used in the production of various therapeutic sera, and they are in good condition when acquired. Guinea-pigs used for experimental work cannot be so carefully chosen, and latent infection may not be evident at the time of the commencement of an experiment. All horses used for the routine production of anti-sera must be protected to a greater or lesser degree against tetanus, and thus it has been possible to use many horses for the comparison of various antigens. The accompanying charts show the percentage of horses with different degrees of immunity response as a result of injection of tetanus toxoid with and without the addition of potash alum. (In our actual tests, the U.S.A. unit of antitoxin was used. For the purpose of this paper the units have been converted into the International scale, *i.e.*, 1 U.S.A. unit = 2 International units).

One Injection.—Charts 1 and 2 show the results of a comparison between the antigenic effect of the same toxoids A and B in horses. Groups of horses were given an initial dose (or primary stimulus) of one of these toxoids with and without the addition of 1 per cent. alum, and after four weeks experimental samples of their blood were tested for antitoxic content. The response to 50 c.c. of the alum toxoid was in both cases considerably greater than when alum was absent ; at the end of four weeks no horse receiving 50 c.c. of the alum toxoid A contained less than six International units of antitoxin per c.c., while in a similar group of horses receiving toxoid A without alum, less than half the horses attained a titre of 0.02 unit per c.c. The weaker toxoid B produced poorer response than toxoid A when alum was added, only 60 per cent. having a value of six or more units ; when alum was absent, no horse showed any detectable antitoxin (less than 0.0004 unit per c.c.) after four weeks ; a similar dose with alum produced 2 units per c.c. in nine horses out of ten. (Charts 1 and 2.)

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In Charts 1 and 2 also, a comparison is made between 10 c.c. and 50 c.c. of these toxoids as a primary stimulus, and although the response to 10 c.c. was less, it was still sufficient for the protection of the horses against tetanus (*i.e.*, an antitoxin content of at least 1/50 unit per c.c.). A stimulus of 5 c.c. did not produce more than 1/50 unit per c.c. in 25 per cent. of the horses injected, while with a stimulus of 1 c.c. the response was definitely too small for the protection of the horses.

Two Injections.—Charts 3 and 4 show the response to a second injection (or secondary stimulus) of 50 c.c. of tetanus toxoid given four weeks after the primary stimulus. Groups of horses were injected with 50 c.c. of the toxoids with and without the addition of 1 per cent. alum in the primary stimulus, and with and without the addition of alum in the secondary stimulus. From the curves, it will be seen that the presence of alum has greater effect in the primary than in the secondary stimulus. Curves I. and II. in both charts indicate this. Curve III., in which alum was present only in the secondary stimulus, shows that ultimate response was not as good as when alum was given initially, though with both toxoids the protection conferred was sufficient. Curve IV., Chart 4, shows that without alum in either stimulus, the response was definitely poor with the weaker toxoid, and far less than the response to a single injection of 10 c.c. of this toxoid with alum, as shown in Curve I., Chart 2. (Charts 3 and 4.)

The response in horses to primary and secondary stimuli each of 10 c.c. of toxoid + alum, was not appreciably less than with stimuli of 50 c.c., all horses having a value greater than 20 units per c.c. when two doses of 50 c.c. were injected, and from 6–20 units when 10 c.c. were injected. With 10 c.c. toxoid B, only 60 per cent. of the horses passed a value of 6 units, whereas when 50 c.c. toxoid B were injected, 95 per cent. contained more than 6 units of antitoxin per c.c.

Comparison between the Immunity Response to the Precipitated Mixtures and the Washed Suspension.

To compare the response to two injections of whole material (toxoid + alum) and of a washed suspension of the alum precipitate, toxoid C, intermediate in value between A and B, was used in groups of about fifty horses. The results of the primary response are recorded in Chart 5, and those of the secondary response in Chart 6. In every horse adequate protection was afforded by two doses, each of 10 c.c., of either material injected at an interval of one month. The best response was obtained by using 1 per cent. alum toxoid as the whole material; after two injections of 10 c.c. at an interval of one month, 100 per cent. of the horses contained more than 1/5 unit, and 90 per cent. more than 6 units. (Charts 5 and 6.)

The object in re-suspending the precipitate is to remove non-specific material likely to cause undue reactions, but the results show that some of the antigenic material is also removed. 100 per cent. of the horses were over 1/5 and 90 per cent. over 2 units per c.c. after two injections of the re-suspended material,

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TABLE 1.

Showing the antitoxic content of guinea-pigs six weeks after the subcutaneous injections of tetanus toxoid, with and without the addition of 1 per cent. alum. (Serum from eight guinea-pigs in a group divided into two pools.)

Toxoid	Index of Combining Power	Alum	Volume injected (c.c.) 0.1	0.2	0.5	1.0	2.0	5.0
			Units of antitoxin (International) per c.c. serum.					
A	0.02	1%	0.2	1.0	1.0	4.0	—	—
			0.2	1.0	2.0	4.0	—	—
		Nil	—	—	—	0.01	0.02	0.2
			—	—	—	0.02	0.1	0.2
B	0.05	1%	0.1	0.1	1.0	0.4	—	—
			0.4	0.2	2.0	1.0	—	—
		Nil	—	—	—	0.004	0.002	0.04
			—	—	—	0.004	0.010	0.04

TABLE 2.

Showing the antitoxic content of guinea-pigs six weeks after subcutaneous injection of tetanus toxoid, with the addition of varying percentages of alum. (Serum from eight guinea-pigs in a group divided into two pools.)

Toxoid	Index of Combining Power	Alum	Volume injected (c.c.) 0.1	0.2	0.5
			Units of antitoxin (Int.) per c.c. serum.		
C	0.03	0.5%	0.002	0.02	0.02
			0.002	0.02	0.4
		1.0%	0.02	0.002	1.0
			0.2	0.10	2.0
		Suspension of 1%	less than 0.0004	0.10	1.0
			0.010	0.2	1.0
		Suspension of 2%	0.004	0.4	2.0
			0.004	1.0	2.0

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but only 65–70 per cent. of the horses had values greater than 6 units. Although in the suspension the whole amount of antigen is not present and the response not quite so good, it is worth while using this form of alum toxoid because the reactions caused by it are smaller.

Conclusions.

Horses may be protected against accidental tetanus by the injection of two doses of tetanus toxoid of suitable strength, at an interval of one month. If 1 per cent. potash alum is added to the toxoid, far greater immunity results, and the majority of horses may be sufficiently protected by a single injection of 10 c.c. of this alum toxoid. The washed re-suspended precipitate produces a lesser degree of reaction when injected, and though some of the antigenic material is removed in the washing process, two doses of 10 c.c. injected at an interval of one month afford adequate protection.

Our thanks are due to Mr. T. Dalling for condensing our original paper into a form suitable for publication in this Journal.

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FOOT-AND-MOUTH DISEASE : SOME PECULIARITIES PRESENTED BY THE DISEASE IN SOUTHERN RHODESIA.*

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It is not the object of this paper to discuss the origin, history and administrative control of foot-and-mouth disease as it exists in S. Rhodesia, but rather to emphasise certain features of it which appear to distinguish it from the foot-and-mouth disease of Great Britain and the Continent of Europe. These peculiarities have been observed not only in the field, but in the laboratory. In the first place the local disease is as a rule exceptionally mild. In many outbreaks it has caused so little disturbance that it has died out, leaving it uncertain as to whether the

* Presented at a Conference of Veterinary Officers at Salisbury, S. Rhodesia, December 10th, 1931.

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majority of the animals have ever been infected. It is probable that many animals contract the disease in a " closed " or " occult " form, that is, in a form characterised by an elevation of temperature without " open " lesions.

Another remarkable feature is the manner in which the disease is spread. I think I am right in saying that in every outbreak the origin of which has been traced, infection has been introduced by infected cattle, and that although the origin of infection in some outbreaks has not been determined, in no instance has it been possible to definitely incriminate any other source of infection. This is entirely different from what obtains in Great Britain and elsewhere, where, as we know, the disease is regarded as one of the most highly infective and most difficult to control. If the virus of the local form of the disease were equally resistant and easily transmitted, the dissemination of infection throughout the country would have been more general. As it is, it would appear that the infected ox, and the ox alone, is the only agent of transmission. Motor cars, vehicles drawn by horses, mules or donkeys, human beings and wild animals, have passed freely from infected to clean areas without disseminating infection. For example, on the Central Estates large numbers of game have moved freely from infected to clean paddocks without carrying infection ; cattle travelling along roads daily traversed by infected herds have not become infected ; cattle grazing on one side of a fence have not become infected from sick animals grazing on the other side. Also stockowners, in order to hasten the progress of infection through their herds, have adopted various methods with a view to infecting their cattle, as, for example, by placing in their mouths material saturated with the saliva of sick animals, by laying down salt to be licked by sick and healthy, by running sick cattle with healthy troops, but with very little success. It is only when they have closely crowded together sick and healthy that they have succeeded in appreciably hastening general infection. Laboratory investigations appear to offer an explanation, and it may now be well to discuss some of the work which has been carried out at the Veterinary Research Station, Salisbury, during the past three months.

It may be explained that so long as the disease was confined to Matabeleland it was thought undesirable to establish a focus of infection in Mashonaland, even for experimental purposes, but when it made its appearance in August in the Salisbury district, and within a few miles of the Veterinary Research Station, these objections no longer obtained. Investigations, however, were thus delayed for five months, and a further three weeks was lost by the unexpected failure to set up the disease in laboratory animals. It will thus be seen that laboratory investigations have only recently been carried out, and that sufficient time has not yet elapsed to enable many important features of the disease to be worked out.

As soon as the disease appeared in the vicinity of the laboratory, material was collected from natural cases of infection. Swabs from typical oral lesions, ruptured and unruptured vesicles were taken, and blood from animals in the thermal stages of infection was collected in amber glass bottles containing appropriate

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solutions of citrate and glycerine. These were hastily brought to the laboratory, and the former were rubbed over the buccal mucous membrane of susceptible cattle. Other swabs were soaked in physiological saline, and after rough filtration a quantity of the liquid was injected into the jugular vein of normal cattle. The citrated blood, with and without glycerine, was similarly injected into healthy cattle. In view of the facility with which the European infection can be transmitted, our failure to set up the disease in susceptible animals with carefully selected material caused considerable surprise, and gave rise to the suspicion that our technique was at fault. Repeated attempts, however, confirmed our first results.

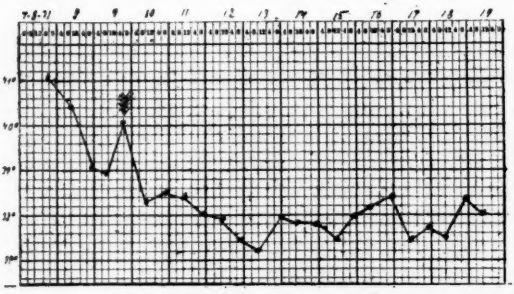

CHART No. 1.—Horse No. 612.

In Hutyra and Marek's "Special Pathology and Therapeutics of the Diseases of Domestic Animals," the facility with which infection can be transmitted from sick to normal animals is dealt with under various headings, and under "emergency inoculation" they describe the technique of inoculation as follows: "The simplest procedure consists in rubbing or superficially scarifying the mucous membrane of the mouth lightly with a clean, coarse piece of linen, and then applying the saliva of affected cattle; or the animals are given coarse food contaminated with such saliva. . . . Likewise very simple and certain is the method in which a piece of linen is placed in the mouth of the affected animal, which is allowed to chew at it for several minutes, then the well-saturated cloth is introduced into the mouth of the next six to eight cattle, and they are also allowed to chew upon it." As has previously been stated, our own repeated efforts to transmit infection

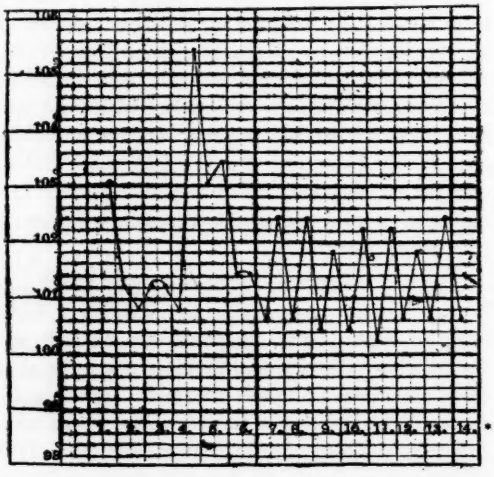

CHART No. 2.—Steer No. 18. Intra-nasal inoculation with blood from No. 10, seven days old.

* = Days.

in a similar manner failed. Loffler and Frosch's method of immunisation consisted in inoculating subcutaneously into cattle virulent lymph from vesicles, yet our own attempts to transmit the disease by subcutaneous and intravenous inoculation of vesicular fluid, or blood, failed.

It was not until infective material was introduced into the nasal cavities of susceptible cattle that infection could be regularly established; but when once this method was adopted the work proceeded rapidly, and many unexpected characteristics of the local disease came to light. It was found that by introducing infective material in this way a thermal reaction was produced, and that if nasal swabs or blood were taken from an animal during the early stages of that thermal reaction, these, when introduced into the noses of other cattle, would again give rise to thermal reactions.

It was for some time uncertain as to whether reactions were caused by foot-and-mouth disease, but after four passages through animals which yielded thermal reactions only, an ox inoculated with blood from a reacting heifer developed characteristic lesions of that disease in the mouth, thus proving the specific nature of the reactions. This observation is of particular interest in that it indicates that animals may suffer from thermal reactions only, without open lesions. Andrews, in the Fourth Progress Report of the Foot-and-Mouth Disease Research Committee, page 78, refers to "a small number of inoculated cattle and pigs" which "have undergone a febrile reaction, accompanied in some instances by signs of systemic disturbance, but without exhibiting any local lesions," and states that "in all cases the animals failed subsequently to react to inoculations of large amounts of the same virus." He adds, however, that "blood taken at the height of the reaction failed to infect when inoculated intramuscularly into susceptible subjects." We have not tried the intramuscular injection of blood taken from our cases, but such blood injected into the noses of susceptible cattle has invariably infected them. We may also mention here that animals suffering from an "occult," or, as we have called it, a "closed" reaction, characterised by thermal elevation only, have during that reaction proved infective to in-contact healthy cattle. This will be discussed later.

Reverting now to the difficulty experienced in transmitting the disease under laboratory conditions, the case of ox No. 19 is of particular interest. This animal developed severe oral lesions after a comparatively mild thermal reaction. Vesicles appeared on the lips and gums, and on manipulation a large portion of the epithelium of the tongue was peeled off. For three days it stood in its stall salivating copiously over its food, water and bedding. On the third day it was removed, and a shorthorn heifer was tied in its place, and was given no food or water until that contaminated by ox No. 19 had been consumed, and it was compelled to stand or lie upon the contaminated bedding. This heifer did not become infected. It is, of course, known that infection will die out somewhat quickly from stables. Leabilly, Vallée, and Carré have drawn attention to this fact, and recently Andrews has confirmed their results. He found that in his experiments "on no occasion was the donor or the contaminated box proved

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infected after the fourth day from the rupture of the vesicles." Up to the fourth day they appeared usually to be infective. But in the case of ox No. 19, it had only been salivating for three days, and the saliva excreted on the first day must have been infected, and probably remained so until the heifer was placed in the stall, and for some days after.

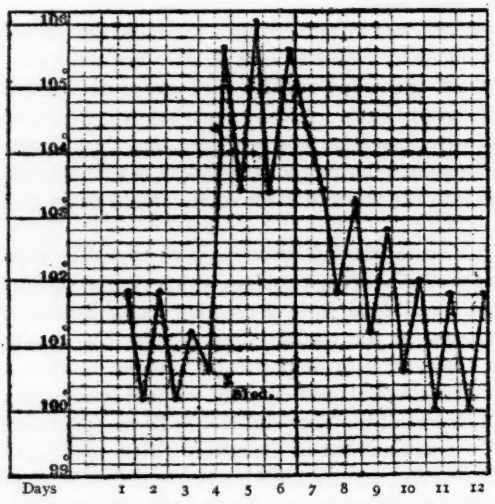

CHART No. 3.—Heifer No. 15.—Blood from this animal taken on fourth day caused " open " lesions in Ox. No. 19.

Another case, No. 14, was compelled to drink from a certain bucket throughout its thermal reaction. The water from the bucket was not removed, but a little was added daily. On the third day, when the animal's temperature was still high, the bucket and the water it contained was placed before heifer No. 21, which was compelled, *faute de mieux*, to drink it. This animal did not become infected. It developed no temperature or immunity, and later was found to be susceptible when inoculated with another virus.

During the course of our experiments it has frequently been observed that when healthy animals have been tied up in a stall side by side with reacting animals, but with their heads some short distance apart, they have not become infected. On the other hand, there is evidence that animals suffering from a thermal reaction without open lesions may infect animals in contact with them, provided such contact is sufficiently close. The following emphasises these points: Nos. 34, 35 and 36 were infected by injecting the blood of No. 27 into their noses. They were allowed to run, during the thermal reaction which followed, in a paddock with Nos. 38, 39, 40, 41, 42 and 43, all of which contracted the disease. Grazing

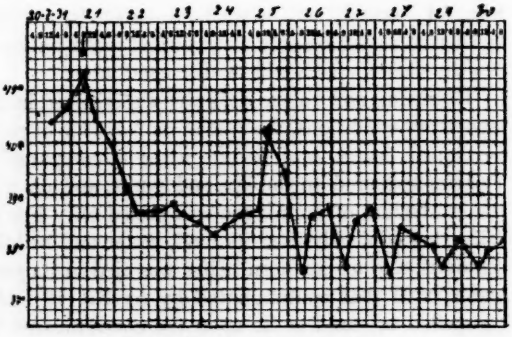

CHART No. 4.—Horse No. 338.

being poor in the paddock, hay and mealie meal were supplied, the latter in a trough from which sick and healthy fed together, coming into the closest " nose-to-nose " contact. But there was also a water-trough common to this, and an adjoining paddock at which the cattle from both paddocks used to drink, yet none of those in the latter paddock became infected. Also six cattle which were in another adjoining paddock throughout the whole period during which those in the first paddock were reacting, and were only separated by a barbed-wire fence, did not become infected. Both laboratory and field evidence, therefore, indicate that infection is not readily transmitted, and that close " head-to-head " contact between sick and susceptible is necessary. It may be of interest here to record that Mr. Borthwick, late Principal Veterinary Officer for the Union of South Africa, verbally informed the writer that during the 1892 outbreak of foot-and-mouth disease, which is said to have originated in this country, the difficulty of transmitting the infection was also encountered, and as the result of experience it was found that the best way of doing so was by the close crowding of animals together in kraals. It is significant that our experience of the present outbreak leads us to the same conclusion.

Apart from the difficulty in transmission, both laboratory and field evidence suggests that the viability of our virus is extremely brief. The remarkable tenacity of the European virus outside the animal body is well known. For example : " virus dried on silk fabric was infective for one, but not for two weeks ; on woollen fabric it was infective for as long as fifteen weeks ; on bran for at least ten weeks ; on cow's hair for four weeks ; on sand for two weeks." In an experiment at Pirbright, hay was contaminated with saliva from cattle during the acute stage of the disease, and stored in sacks. One bovine was infected by feeding hay that had been stored for four weeks. And yet at this laboratory heifer No. 24, which ate the food and drank the water contaminated by ox No. 19, and No. 21 which drank the water contaminated by No. 14, and six cattle which drank the water from the trough from which nine infected animals were compelled to drink during their reactions, did not become infected. Recently Waldmann and Reppin have reported that " in cattle the saliva may contain virus as early as nine hours after experimental infection, and before there are any macroscopic changes in the tissues of the mouth. The virus was present in the saliva for as long as six days p.i., and in bits of epithelium which were being shed from the vesicles of the mouth for as long as eleven days." If, therefore, the local virus had been as viable as the European virus with which the above results were obtained, it might have been expected to give rise to infection in at least some of our own cases where every facility for infection was present. The survival of the local virus within and without the animal body is a matter of greatest importance from the administrative point of view, but it has not been possible in the brief period during which we have been able to investigate the matter to deal satisfactorily with these questions. Our own experience up to the present concerning the viability of the Rhodesian virus outside the body has been limited

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to observations concerning the infectivity of blood from infected animals. Such blood was in all cases taken from the jugular vein of infective cattle, into citrate solution one part, glycerine one part, the blood constituting the remaining third part. Glycerine was added as a preservative in conformity with the technique of Arkwright and Burbury, and other workers.

It will be seen from the accompanying table that blood from No. 10 proved infective when introduced into the nose of No. 12 on the day of collection, also infective after seven days, but caused doubtful if any reactions when similarly introduced after fourteen days and twenty-one days. Virus 35 proved infective after two days, but not after ten days. Virus 19 proved infective after eight days, and the Teneriffe virus, whether with glycerine or without, proved inert after seventeen and twenty-two days. Virus No. 44 and No. 45 proved negative after ten days. Thus the longest period of viability in blood preserved in glycerine-citrated saline solution was eight days. It is of interest to compare these results with those obtained by European observers. Lebailly, for example, kept citrated blood from an infected animal for seventy-two days; and in the fourth report of the Foot-and-Mouth Commission, page 81, Andrews mentions a case of an animal slaughtered when the virus was in fair concentration, but could no longer be demonstrated after preservation of the blood for fourteen days at 29° F., but specifically mentions that this is a " curious and unusual observation." Again, on page 51, it is recorded that blood taken nine days after the animal's death showed a comparatively high virus content.

The virus is found in largest concentration free in the blood serum of an infected animal, and outside the body its viability largely depends on the substrata upon which it is dried. Thus it survived in blood dried on leather hides and rubber at 12–20° C., from 80 to 102 days, and up to 40 days at 29° F. in meat-wrapping cloths soaked in virulent blood. Also up to eighty days at 29° F. in bone-marrow and blood-clot. In another report it is stated that the infectivity of a carcase largely depends upon the virus in the blood it contains. If the European virus is so long-lived in citrate and in dried form, the brevity of the viability of the Rhodesian strain under what should be favourable conditions for preservation rather points to a " distinction with a difference."

The duration of infectivity of the affected animal is also a matter of considerable importance, which time has not yet permitted us to work out. Experience in the field indicates that it is comparatively brief, and laboratory cases point to the same conclusion. For example, oxen Nos. 34 and 35 were inoculated on October 22nd, and developed " closed " reactions from five to eight days after; Nos. 36 and 37, inoculated the same day, developed similar reactions a few days later. From these, Nos. 40, 41, 42 and 43 contracted the disease probably during the early thermal periods. Three of them developed " open " lesions. Six healthy animals turned into the paddock with these eight animals on November 24th, and running with them continually for more than three weeks, did not become infected.

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The statement by Waldmann that by special method of examination of the blood and urine of 500 cattle, the virus had been found in the blood of 2.6 per cent. of them on and after the seventh day, and that in eight of those whose blood contained virus, virus was present in the urine from six of them at varying intervals from the sixth to the two hundred and forty-sixth day after infection, although it was not excreted in urine at the same time as it was present in the blood, is disconcerting in that it gives excuse for the prolongation of the embargo upon our cattle.

It was only in August last, at the Eleventh International Veterinary Congress, London, however, that the same observer stated: "As we know that

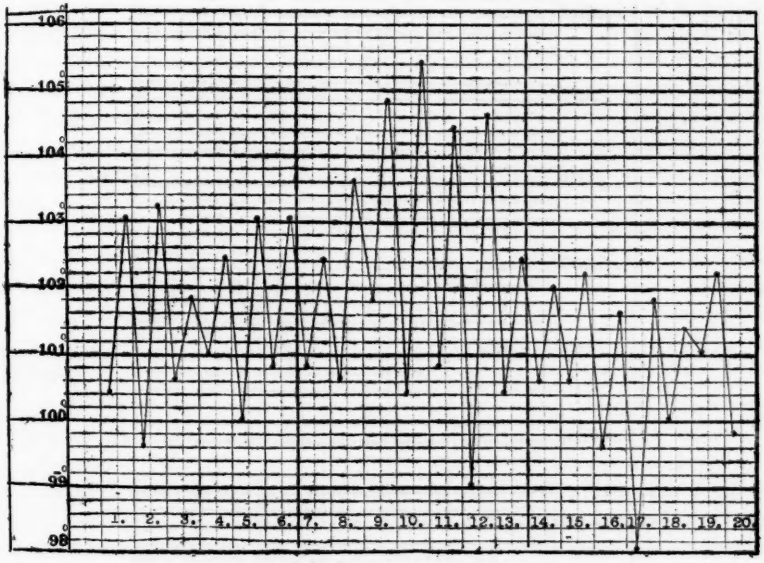

CHART No. 5.—Heifer No. 42. Infected by contact with No. 35, which suffered from thermal reaction only.

* = Days.

the virus is no longer excreted after eleven days after infection, we consider the danger to neighbouring premises to be over after that interval, and allow relaxations in the control measures to follow forthwith." And since, according to him, "millions of cattle have been inoculated in the last few years in Germany" by a simultaneous virus-serum method which actually gives rise to a controlled infection, his scientific observations do not appear to be in accord with the general practical experience. Moreover, it appears that his experiments were carried out with more tenacious strains of virus than that obtaining in S. Rhodesia. He mentions that in shed epithelium his virus was very tenacious, and could remain virulent in the open for 67 days (Trantwein), in water 41 days, in dry

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stable dust 43 days, and that it resists decomposition in epithelium in stagnant water for 39 days in summer, 49 days in autumn, and 103 days in winter. It is significant, therefore, that even with such a tenacious virus to control, the German authorities have considered it safe to relax control measures eleven days after infection, and it will be interesting to note whether as the result of Waldmann's recent observations the period of quarantine will be extended from eleven days to eight and a-half months.

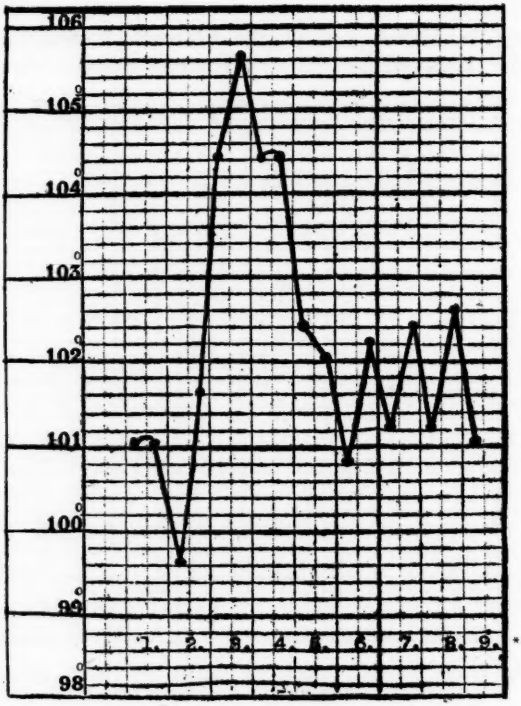

CHART No. 6.—Heifer No. 10. Intra-nasal inoculation with swabs from Crowborough cattle. Example of short sharp reaction.

* = Days.

It is clear from field observations that under certain conditions the virus becomes modified by passage. Apparently it can become exalted or attenuated according to the susceptibility or resistance of the host. At Liebig's ranch we are told " the mortality amongst pure bred Sussex calves was less than 10 per cent., amongst Aberdeen-Angus and Africander calves it was very much less." And in certain local outbreaks the symptoms among calves were negligible. As previously stated, in one outbreak it was only by catching and carefully examining the calves that it could be determined whether or not they were infected. It

appears to be the general opinion that better-bred animals suffer more severely than native or indigenous animals. Our laboratory experience has been that a virus which throughout a series of passages may produce only thermal or " closed " reactions may by passage through a highly susceptible animal resume the power of producing " open " lesions. Our experience is limited, but one is inclined to the view that passage through a " soft " fat, well-bred animal may increase the virulence. This is in accord with our experience in connection with the method of inoculation of horses against the local form of African horse sickness by means of an attenuated virus, which in animals of that type becomes exalted. The mortality is almost entirely limited to such animals.

From observations which have been made in the laboratory and in the field, there appears good reason to believe that the foot-and-mouth disease as it occurs in this country differs in many respects from that met with in Great Britain and the Continent of Europe. For example, it appears to be of very low infectivity and difficult to transmit. The viability of the virus outside the animal body appears to be exceptionally brief, and recovered animals do not appear to remain infective after recovery. All these characteristics render the disease comparatively easy to control, and justify the hope that it will soon be completely eliminated. It has to be admitted, however, that the research work which up to the present has been possible, is very desultory and incomplete. Much more remains to be done. The nature of the virus should be ascertained, and the various strains of which it is composed should be " typed " by modern biological methods. It would be of scientific and practical interest to determine whether it is of European type or whether it is an African, or perhaps a local virus *sui generis*.

It might be found to be a European virus attenuated in local cattle, or an African virus enzootic in certain animals and in certain districts, after the manner of " Snotziekte " and African swine fever. It is of the utmost importance also to ascertain definitely the infectivity and viability of the virus and its natural methods of transmission, for upon such knowledge administrative measures of control must depend.

INVESTIGATIONS CONCERNING POULTRY TYPHUS AND WHITE DIARRHŒA IN CHICKENS.

By P. I. STENIUS.

State Veterinary Laboratory, Helsingfors, Finland, Dr. R. Hindersson, Director.

THE " Pullorum disease " is caused by *B. pullorum*, or, as it is more rightly called, *B. gallinarum-Klein*. Identical names are *B. sanguinarium*, *B. paradysenteriæ gallinarum*, etc. The illness was first described in 1889 by Klein, England, who observed among full-grown poultry an extraordinarily virulent illness causing great mortality. He succeeded in proving bacteriologically that this was caused by a bacterium belonging to the paratyphus group. Moore

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reports, in 1895, an outbreak of illness among full-grown poultry in U.S.A. This illness agreed entirely with that described by Klein. In 1900, Rettger noted mass mortality among chickens in several chicken farms in North America. The full-grown poultry were, generally speaking, immune from this disease. To begin with, it was believed that the enzootic described by Rettger was a separate disease which had no connection with that described by Klein. Manninger, Lerche, Christiansen, Haupt and others have, however, shown through very thorough tests that these diseases are identical, although several varieties of *B. gallinarum* are pathogenous to chickens, others again to full-grown poultry.

Besides poultry, ducklings are also susceptible to the pullorum contagion. Whether or not full-grown ducks can be infected, is not yet fully proved. Geese are believed to be immune from this disease. Rabbits and guinea-pigs are easily infected when inoculated.

The pullorum disease is considered in America as well as in Europe to be the most dangerous of all poultry diseases. Every year innumerable chickens succumb to this disease, and also among full-grown poultry besides isolated cases, quite severe enzootics have been noted. It is possible for the chickens to be infected while still in the egg. In such cases the chicken often dies before being hatched, but even if the infected chickens are hatched alive, their powers of resistance are so undermined that they usually die within a few hours. The chickens are most susceptible to infection during the first few days after being hatched. Chickens one or two days old which catch the pullorum disease show the following symptoms : the sick chickens separate themselves from the healthy ones, and seek some hidden corner where they crouch, their wings drooping, now and then emitting a weak piping sound. The excrement is either a yellowish-green or chalk-white in colour. The chalk-white colour is caused by great quantities of uric acid crystals. Diarrhœa is not always observed. In such cases, which are not at all rare, the disease takes a septicæmic course. The older the chickens are, the greater are their powers of resistance. Chickens a few weeks old often get the disease only in quite a mild form. The symptoms are as follows : ruffled plumage and anæmic mucous membranes ; diarrhœa is often observed, although not in all cases ; the chickens show great weakness. According to Lerche, paralytic symptoms are by no means infrequent. The bird rests in this case on the lower part of its legs, counting from the shank joint downwards. The chickens which have recovered from the illness (in most cases older chickens) remain bacillus carriers, and harbour the contagion in the follicles of the ovary. The bacillus carriers are, of course, a continual danger to the poultry farm. They usually show no symptoms of disease, and even the ability to lay eggs is in most cases good. Under unfavourable conditions, *i.e.*, when the poultry, through neglect or other outside influence, have less resistance, the bacillus carriers can fall ill with blood poisoning. The disease can then become enzootic. In this case even the full-grown poultry are susceptible to the disease. Usually, however, the pullorum disease appears

either only amongst chickens or amongst full-grown poultry, depending on the contagion being pathogenous to the former or the latter. It should be noted that in most cases those stock strains of bacilli which are harboured by the bacillus carriers in the ovary are chicken pathogenous, and thus under normal conditions are not of danger to full-grown poultry.

Full-grown poultry which have been infected by the pullorum disease show the following symptoms : The comb is cyanotic and the mucous membranes anæmic ; the wings droop, and the plumage is ruffled. A not unusual complication of the disease is ascites. Most often the illness appears particularly acute among full-grown poultry. Many cases have been recorded where the poultry have died without any preceding symptoms of the illness. Less virulent and chronically developing cases of the disease occur now and then. The pullorum disease varies considerably as to mortality. Thus in certain cases practically all the chickens in an infected stock could succumb to the disease, while in other cases most recover from it, or on the whole are not susceptible. The mortality can thus vary from 2–90 per cent., and even up to 100 per cent., among chickens. In the worst cases mortality among full-grown poultry can amount to 20 per cent. These data confirmed abroad, agree quite well with the results arrived at through research work done by the veterinary laboratory here.

The pathologic anatomic changes during the pullorum illness are quite typical. One observes that the chickens have grey or yellow nodes of the size of a pin's head, or larger, in the liver, in the lungs, and in the heart muscles. The liver and spleen are somewhat swollen, and a degeneration of the liver parenchyma is also to be found. Christiansen considers the changes in the lungs to be the most typical signs of the pullorum disease. Strangely enough, the intestinal changes are quite minimal. Often only a slight swelling and congestion of blood in the mucous membrane can be observed. The contents of the intestines are slimy and yellowish in colour. An especially important factor in white diarrhœa is the retention of the *vitellus nutritivus* in chickens. If this is not absorbed, but occurs quite extensively in the abdominal cavity, there is every reason to suspect white diarrhœa.

With full-grown poultry the pathologic anatomic changes are somewhat different. Degeneration of the liver, with numerous subcapsular bleedings and greyish or yellow miliary nodes. Even rupture of the liver often occurs, and death follows through inner bleeding. Also in the lungs greyish or yellow miliary nodes can be observed. Ascites combined with fibrinous peritonitis is very often found in the abdominal cavity. This is frequently combined with a fibrinous pericarditis. The most typical changes, however, are to be observed in the ovary. The infected follicles are often of a firm consistence, and vary considerably in colour. Thus they could be of a grey-yellow, grey-green, or brown colour. In shape they are singularly irregular, with bumpy and angular surface. The contents are yellowish or brown, either curdled or liquid. Similar changes can be observed in the ovary in nearly every case of latent pullorum infection in full-

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grown poultry. Inflammation of the intestines is quite common in full-grown birds, but even among these it is by no means always present. Lastly, it may be mentioned that the pullorum bacterium, although rarely, can cause a fibrinous inflammation of the oviduct.

Chickens and poultry dissected at the veterinary laboratory which have died of the pullorum disease have, as a rule, shown changes typical of the illness. In isolated cases, however, no pathologic anatomic changes have been observed in chickens, although the bacteriological investigation has been positive. Bacterium pullorum is one of the bacteria belonging to the coli paratyphus group. It lacks the power to move, and is somewhat thinner than the *Bacterium paratyphus* which it morphologically most nearly resembles. In older cultures it often grows into long threads. The *Bacterium pullorum* shows itself to be singularly variable under culture. On agaric it forms after forty-eight hours scanty bluish colonies. By the addition of serum it grows more profuse. It has been noted that different stock strains show themselves to be quite different in regard to the manner of growth, thus Manninger mentions that he cultivated pullorum bacteria that grew in profuse colonies more nearly resembling those formed by the *Bacterium paratyphus*. Every isolated stock strain (altogether about thirty or forty) at the veterinary laboratory have on agaric, with the addition of a small quantity of serum, formed scanty bluish colonies with sharply defined edge. On Drigalski-agaric and on gelatine the *Bacterium pullorum* forms about the same colonies as on agaric. *Bacterium pullorum* does not melt the gelatine. In litmus milk the *Bacterium pullorum* forms a red colour. Because the poultry typhus bacterium usually gives the litmus milk a blue colour, it has been believed that they have been separate bacteria, but Manninger, Lerche and others have isolated both poultry-typhus and pullorum strains which have had contradictory qualities.

TABLE SHOWING THE GAS AND ACID PRODUCING QUALITIES OF TEN DIFFERENT PULLORUM STRAINS IN DIFFERENT KINDS OF SUGAR.

Stock strain	Dextrose		Maltose		Laktose		Sacharose	
	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid
Antigen	+	+	—	+	—	—	—	—
No. 26609	+	+	—	+	—	—	—	—
No. 4209	+	+	—	+	—	—	—	—
Hartwall	—	+	—	+	—	—	—	—
Wien	—	+	—	+	—	—	—	—
Melasniemi	—	+	—	+	—	—	—	—
Vanha-lapio	—	+	—	+	—	—	—	—
Thuren	—	+	—	+	—	—	—	—
Vanninen	—	+	—	+	—	—	—	—
Viertola	—	+	—	+	—	—	—	—

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Based on the reaction of different pullorum stock strains to different kinds of sugar, these have been classed as pullorum-A, and pullorum-B. The poultry typhus bacterium is considered to be as third variety of *B. pullorum*. According to Manninger, those pullorum bacteria which in dextrose, and possibly in mannos and arabinos, form gas, are classed in pullorum-A. Pullorum-B and the poultry typhus bacterium form no gas in any kind of sugar. Manninger has, however, proved that gas-forming pullorum bacteria can lose this quality when kept a long time in the laboratory. He has also isolated the poultry typhus bacteria which have had the power to form gas in dextrose. At the veterinary laboratory ten pullorum strains have been tested as to the above mentioned qualities, when it has been shown that two Swedish strains (antigen strain and No. 2669) and one Finnish (4209) formed gas in dextrose. The rest, among them one strain from Vienna, were not gas-forming. Hadley maintains that one can distinguish *B. pullorum* from poultry typhus strains, because *B. pullorum* lacks the power to form acid in maltose, dextrin and dulcit, whereas this quality should be characteristic of the poultry typhus bacterium. Manninger maintains, however, that several pullorum strains also have the ability to form acid in the above-mentioned three kinds of sugar. Investigations at the veterinary laboratory have shown that at least some pullorum strains form acid in maltose. In cases where pullorum bacteria cannot be serologically separated from poultry typhus bacteria, there is no reason to regard these bacteria as two separate kinds. From several quarters it has, therefore, been maintained that for these different pullorum varieties, one name should be chosen, and *Bacterium gallinarum-Klein* has been considered the most suitable because the disease was first described by the Englishman Klein.

Bacterium pullorum occurs in chickens frequently in all the pathologically changed organs. On the other hand, it is comparatively seldom to be found in the blood. In most cases one can easily cultivate the *Bacterium pullorum* from the vitellus nutritivus left in the abdomen. The bacteria can also be found in great quantities in the lung nodes. In the case of full-grown poultry one can find the pullorum bacteria especially numerous in the changed follicles in the ovary. In this way sixteen pullorum strains were cultivated at the veterinary laboratory from nineteen fowls reacting to the pullorum disease which were at the disposal of the laboratory. Cultures were started in every case from the typically changed follicles. In three cases the cultivation was unsuccessful. Lerche and others have had the same experience during their investigations. It should also be mentioned that in none of these nineteen cases has it been possible to cultivate the *Bacterium pullorum* direct from the blood. These birds were all so-called bacillus carriers, that is to say, poultry that harbour contagion in the ovary without suffering anything themselves from this. In poultry suffering from the pullorum disease, *i.e.*, poultry infected with the *Bacterium pullorum*, and showing distinct symptoms of the disease, the contagion occurs in great quantities in all organic changes. From the blood, however, the *Bacterium pullorum* could in only rare cases be cultivated.

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TABLE SHOWING THE SERUM TITRE IN THE CASES OF NINETEEN BIRDS REACTING TO THE PULLORUM DISEASE, WITH THE RESULT OF THE PATHOLOGIC ANATOMIC AND BACTERIOLOGICAL INVESTIGATIONS.

Bird No.	Titre	Cultivation of *B. pull.*	Path. Anat.	Results
118	0.0005	Neg.	Moderately well fed	A few hardened follicles in the ovary
85	0.002	Pos.	Very well fed	Numerous hardened follicles in the ovary
182	0.002	,,	Very well fed	All follicles in the ovary hardened
582	0.005	,,	Moderately well fed	A few hardened follicles in the ovary
297	0.005	,,	Underfed	Well-developed as well as numerous hardened follicles in the ovary
192	0.005	Neg.	Very well fed	Numerous hardened follicles in the ovary
426	0.005	Pos.	Very well fed	Numerous hardened follicles in the ovary
101	0.005	,,	Moderately well fed	A few hardened follicles in the ovary
480	0.005	,,	Well fed	Several hardened follicles in the ovary
177	0.005	,,	Well fed	A few hardened follicles in the ovary
568	0.005	,,	Moderately well fed	A few hardened follicles in the ovary
521	0.01	,,	Very well fed	A few hardened follicles in the ovary
146	0.01	Neg.	Moderately well fed	A few hardened follicles in the ovary
368	0.01	Pos.	Very well fed	A few hardened follicles in the ovary
603	0.01	,,	Well fed	A few hardened follicles in the ovary
496	0.01	,,	Very well fed	Numerous hardened follicles in the ovary
996	0.01	,,	Very well fed	A few hardened follicles in the ovary
67	0.02	,,	Well fed	Numerous hardened follicles in the ovary
414	0.02	,,	Well fed	A few hardened follicles in the ovary

In investigating the occurrence of pullorum bacteria in eggs originating from poultry suffering from the pullorum disease, very varying results have been obtained abroad. Thus some scientists have found that only about 2 per cent. of all examined eggs have been infected, while others again arrived at a much higher percentage, in some cases even up to from 20–50 per cent. To ascertain to what extent the above data agree with the conditions here, a cultivation test of *B. pullorum* from eggs from infected stock was arranged at the veterinary laboratory. Three eggs from each of 100 reacting hens were examined. The eggs were divided into three groups so that one egg from each fowl was placed in each group. The first group was examined immediately, while group No. 2 was kept five days, and group No. 3 ten days in thermostat (37° C.), after which the eggs were examined. Before starting the cultures the eggs were treated for thirty minutes with a solution of sublimate 1 : 1,000. The shell was opened at the broad end of the egg by means of a sterilised knife, after which a small quantity of the yolk was removed with a Pasteur pipette. Cultures were then started on serum agaric plates, and in serum bouillon. The first group were completely sterile, while in groups Nos. 2 and 3 respectively, one egg from each

group was found to be infected. After inspection it was confirmed that both the eggs came from the same bird. The material for investigation is, however, quite too small for definite conclusions to be derived from it. So much, however, should one be able to gather from these investigations, that infected hens very rarely lay infected eggs, and that the pullorum contagion thus, really first after hatching, spreads among the chickens. The diagnosis of the pullorum disease among full-grown poultry can be carried out especially effectively serologically. In America, where the disease has been most serious, extensive investigations have been made by means of the agglutination method. In regard to the reliability of this method, reports varied considerably to begin with. Nowadays, however, the general opinion is that the agglutination test is the most important method in the fight against the pullorum disease. There are three separate methods of agglutination, *viz.*: (1) The tube method; (2) the rapid method I. (serum+antigen); and (3) the rapid method II. (drop of blood+antigen).

In carrying out the tube method, one uses standard antigen as test fluid, *i.e.*, a silting up of *B. pullorum* in physiological solution of salt, with an addition of 0.5 per cent. carbolic acid. The standard antigen is always of a set thickness. The pullorum culture should be silted up immediately it is taken from the thermostat, where it has been allowed to grow for forty-eight hours. The test fluid keeps at the most two weeks, after which new antigen should be made. The agglutination is carried out now in the usual manner, *i.e.*, by serum that must be examined and put into a number of tubes, 0.1, 0.05, 0.02, 0.01 c.c., etc., after which 1 c.c. test fluid is added. In this way the dilution receives 1 : 10, 1 : 20, 1 : 50, 1 : 100, etc. A total agglutination in the tube 1 : 50 is considered a positive result, or perhaps rather not before tube 1 : 100. A hen that gives an agglutination of this kind is surely infected. In judging the agglutination results, one should carefully see that there is no lipoid sediment. The hen's blood contains, namely, under certain circumstances, lipoid substances in great quantities, and these are precipitated in agglutination. If the test fluid in spite of congelation is not clear, lipoid sediment should always be suspected. By keeping the fowl under a fasting cure before the blood test is made, one can avoid a surplus of lipoid substances in the blood. In order to ascertain to what extent the agglutinative substances at different periods can disappear from the fowl's blood, three investigations according to the agglutination method, with a month between each, were made at the veterinary laboratory, on twenty-nine positive and negative reacting fowls from infected poultry farms. From the above Table it appears that agglutinated particles in most cases occur quite regularly in the blood of infected poultry. Foreign scientists, however, mention cases where they have observed that poultry reacting positively have occasionally given negative results.

It has even been observed that the titre varies somewhat in the beginning and at the end of the laying period.

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TABLE SHOWING AGGLUTINATION RESULTS (SERUM RAPID METHOD) ON EXAMINATION OF POULTRY AT THE OCCURRENCE OF AGGLUTINATED PARTICLES IN THE BLOOD AT DIFFERENT PERIODS.

Hen No. ...	182	177	521	603	368	85	480	996	192	146
Exam. No. 1 ...	++	++	++	++	++	++	++	++	++	++
,, No. 2 ...	++	++	++	++	++	++	++	++	++	++
,, No. 3 ...	++	++	++	++	++	++	++	++	++	++

Hen No. ...	297	496	582	118	101_1	568	414	67	101_2	150
Exam. No. 1 ...	++	++	++	++	+	+	+	+	—	—
,, No. 2 ...	++	++	++	++	+	+	+	+	—	—
,, No. 3 ...	++	++	++	+	++	++	+	+	—	—

Hen No. ...	16	142	401	382	47	397	783	205	9
Exam. No. 1 ...	—	—	—	—	—	—	—	—	—
,, No. 2 ...	—	—	—	—	—	—	—	—	—
,, No. 3 ...	—	—	—	—	—	—	—	—	—

Positive pullorum sera from poultry give a quite varying titre. Thus nineteen of these were examined at the veterinary laboratory as to their agglutinative capacity. As test fluid, a standard solution from one of the antigen strains was used. Of these nineteen sera, three gave very high titre (0.0005–0.002) while two gave a relatively weak reaction (0.02). The remaining sera gave an average titre.

Different pullorum strains vary quite considerably during agglutination experiments. At the veterinary laboratory six weak and strongly reacting pullorum strains were tested with weak positive sera. The weak reacting strains gave negative results, whereas of the strong reacting strains some gave distinct, others indistinct reactions. This circumstance is of importance in the choice of pullorum strains for producing test fluid. In 1927, Runnels, Coon, Farley and others introduced the so-called rapid method in the diagnosis of pullorum disease among poultry. This method enables one to make the agglutination test quickly, and at the same time reliably.

To begin with, the test proved to be not quite reliable, but the more it was developed the surer the results became. This method has been called by the above-mentioned scientists, " rapid method for the diagnosis of the pullorum disease." The rapid method has later been simplified by Bunyea, Hall and Dorset, so that the diagnosis can now be made in the poultry farm.

Runnels, Coon and Farley describe their original method thus: A small square case which is painted black inside is used. One or more electric lamps are fitted inside the case (several because they give a more even warmth). These lamps warm, and throw their light on to a glass plate which forms the lid of the case. The glass plate is divided into squares of 2 cm. The test fluid consists

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of freshly silted up bacteria in a solution of 12 per cent. common salt, with an addition of 0.5 per cent. phenol. The density of the silt should be fifty times greater than the density of the standard antigen. One takes up with a pipette 0.02 ccm. of the poultry serum which is to be examined, and puts it in a square

TABLE SHOWING THE AGGLUTINATIVE CAPACITY OF A NUMBER OF DIFFERENT PULLORUM SERA.

Reacting Pull. poultry.	Serum doses of different strengths. 0.05	0.02	0.01	0.005	0.002	0.001	0.0005	0.0002
118	+	+	+	+	+	+	+	—
85	+	+	+	+	+	—	—	—
182	+	+	+	+	+	—	—	—
582	+	+	+	+	—	—	—	—
297	+	+	+	+	—	—	—	—
192	+	+	+	+	—	—	—	—
426	+	+	+	+	—	—	—	—
101	+	+	+	+	—	—	—	—
480	+	+	+	+	—	—	—	—
177	+	+	+	+	—	—	—	—
568	+	+	+	+	—	—	—	—
521	+	+	+	—	—	—	—	—
146	+	+	+	—	—	—	—	—
368	+	+	+	—	—	—	—	—
603	+	+	+	—	—	—	—	—
496	+	+	+	—	—	—	—	—
996	+	+	+	—	—	—	—	—
67	+	+	—	—	—	—	—	—
414	+	+	—	—	—	—	—	—

+ = distinct agglutination. — = no agglutination.

on the glass plate. One adds to this 0.02 c.c. test fluid, and mixes the two fluids together thoroughly with a platinum needle. If the serum is positive, the agglutination commences in most cases immediately. If the serum is weak, the agglutination is somewhat delayed. This can be hastened by shaking the case. To ascertain whether the preparation of the test fluid could be made more effective, agglutination tests were made at the veterinary laboratory with test fluids of different density, and containing different quantities of salt. It was in this way established that a density of fifty times the density of the standard antigen was the most suitable. On the other hand, no difference could be ascertained if the test fluid contained 0.85 per cent., 5 per cent., or 12 per cent. salt. Dorset maintains that 0.85 per cent. solution of salt without any addition of phenol should be the most suitable to be used for the silting up of bacteria cultures, but no advantage from such a procedure could be noticed at the laboratory.

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It is, however, important that the test fluid should not be older than two weeks at the outside.

The rapid agglutination test, according to the Dorset method, is made in the poultry yard. Also in this case a similar box, as in the previous method, is used. The blood that is needed is obtained through a small cut in the comb of the hen. A drop of the blood is spread on the glass plate, and 0.02 ccm. of the fluid added. Both the blood and the test fluid should then be well mixed. The agglutination commences immediately. If one is not in a position to make the test on the spot, a few drops of blood can be applied to a piece of blotting-paper, and sent to the laboratory for examination. The blood is dissolved at the laboratory for thirty minutes in 0.5 ccm. physiological solution of salt. Five drops of the fluid thus obtained are spread on the glass plate, and one drop of the test fluid added and well mixed. Agglutination commences immediately.

According to tests made by Miessner respecting the reliability and practicability of the above three methods of agglutination, the method, according to Runnels (*i.e.*, when the serum + test fluid are mixed together on a glass plate), should be the most sure, and at the same time more practical than the tube method. The veterinary laboratory investigations have proved that the serum rapid method is considerably more sensitive than the tube method. Also wrong conclusions as a consequence of lipoid sediment are in this way avoided. On the other hand, one observes now and then in the serum rapid method congealing of the test fluid, although pullorum infection is not present. Such non-specific reactions are often especially difficult to distinguish from the real reactions. It is quite natural that strongly agglutinative sera give a typical reaction. In doubtful cases, however, one has no other resource than to make a bacteriological examination of the suspected poultry.

In America they have lately adopted the Dorset method to a great extent in the verifying of pullorum infection. When this method, however, showed itself to be less sensitive than the other methods, an investigation was made at the veterinary laboratory for comparing the Dorset method with the serum rapid method. To begin with, the nineteen birds belonging to the laboratory which had given distinct positive reactions with the serum rapid method were also examined according to the Dorset method. All the birds reacted typically. Besides these, five poultry farms in the Tavastehus district were investigated. The result of the investigation is shown in the following table :

AGGLUTINATION RESULTS ACCORDING TO THE SERUM RAPID METHOD AND THE DORSET METHOD.

	Number of examined cases.	Reactions according to serum rapid method.	Reactions according to the Dorset method.
Poultry farm I	310	6	6
Poultry farm II	278	5	5
Poultry farm III	170	12	5
Poultry farm IV	289	29	27
Poultry farm V	349	166	156

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Although considerably more positive reactions have been arrived at by the serum rapid method than by the Dorset method, as is plainly shown by the table, it has been observed in rare cases that according to the serum rapid method, especially strongly reacting poultry sera have, according to the Dorset method, given negative results. It should be mentioned that in two cases of positive reaction according to the Dorset method, the serum rapid method has failed us. This last-mentioned circumstance does not, however, prevent one from considering the serum rapid method considerably more reliable than the Dorset method. The Dorset method, however, is of great practical importance because it can be carried out at the poultry farm.

Miessner has declared that the Dorset method could in all probability be introduced into practice, so that the veterinary surgeon himself could carry out the investigation. I consider that this is at present out of the question. To avoid wrong conclusions it is necessary that the examination of sera is done at a centre where sufficient experience and technical knowledge is at hand for arriving at correct conclusions.

When making the agglutination test it should be noted that certain conditions can have a not immaterial influence upon the result. Thus it should be noted that young hens which have not formerly reacted can suddenly show a high titre when they begin laying. Likewise one always obtains a greater number of reactions at the end of the laying period than at the beginning. It is also important that positive reacting hens can occasionally, although very seldom, give negative results. It does not always mean by this that they have ceased to be bacillus carriers. Positive poultry sera after being kept a long time (about two weeks) lose their agglutinative power. On what this circumstance depends is at present not known. It does not seem to have any connection with any processes of decomposition, although these would hasten the serum becoming inactive.

In judging the pathogenicity and virulence of the pullorum bacterium in different poultry stocks, many circumstances should be taken into consideration. Highly cultivated stocks, and those weakened by in-breeding, are naturally considerably more susceptible to the ravages of disease than ordinary country breeds. Badly ventilated, damp, and unhygienic buildings, neglect, unsuitable food, etc., encourage to a great extent the activity of the disease.

It should also be taken into consideration that chickens which are accustomed to certain surroundings and conditions, if removed to others, although even better, can fall seriously ill, though they have not earlier shown any symptoms of disease. Moreover, some pullorum stock strains are more contagious than others. The virulence is, however, not always unchangeable, but varies considerably, depending on whether the possibilities for the bacteria to develop are favourable or not. By paying attention to these circumstances, and with the help of repeated serological examinations, one should gradually be able to eliminate the sick poultry from the healthy in an infected stock. This procedure is often exceedingly trying to the patience both of the owner and the veterinary surgeon, but the work should though in most cases be crowned with success.

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ON THE DIFFERENT FORMS OF CATARRH IN POULTRY.

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(1) Simple Catarrh (Colds).

SIMPLE catarrh is a mild inflammation of the mucous membranes covering the nasal passages. No definite cause can be assigned to this affection, but it usually attacks weak birds exposed to unfavourable conditions, such as exposure to cold, draughts, rain, dampness, non-hygienic houses, and improperly nourished birds. All these conditions may be regarded as predisposing causes, and tend to lower the resistance of the mucous membranes of the nostrils to the various organisms normally present there.

Nasal catarrh occurs usually in periods during which there is a wide and sudden variation in temperature during the day and night, as in spring and autumn. It is common for young birds that are infected with intestinal worms to be affected with colds.

Symptoms.—Usually it is easy to spot the sick birds even from a distance, as their nostrils are covered with dust and dry food which stick to the discharge. The affected bird is more or less dull in appearance, according to the severity of the attack. The appetite is diminished, breathing becomes difficult, there is sneezing, and a watery discharge from one or both nostrils. This discharge may disappear in from two to four days, or it may take on a viscid consistency, closing the nasal openings and necessitating breathing through the mouth. At times the discharge may escape through the mouth by way of the cleft found in the upper part of the mouth of the fowl. In some cases there is also lacrimation.

Treatment.—Sick birds should be removed to a clean, warm place, and should be provided with better conditions. In more severe cases the nostrils should be cleaned by washing them well with saline solution containing 1 per cent. sodium bicarbonate, and then well disinfected with one of the following solutions: 2–3 per cent. boric acid; 1 per cent. creoline, 0.5 per cent. potassium permanganate, or a drop or two of tincture of iodine may be applied to each nostril,

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and to the cleft found in the upper part of the mouth. The eyes should be first washed with salt water and a few drops of boric acid instilled.

The food of sick birds should be clean and free from dust, and easily digested, such as : bread soaked in milk, mashes in milk, sour milk and vegetables. Potassium permanganate may be added to the drinking water in sufficient quantity to produce port-wine colour. The drinking water should not be too cold, as in the early morning, as the body of birds at these hours is warm. The houses should be carefully inspected for holes and cracks, which should be repaired, and to see that the number of birds is not too large for the houses, especially during the night. Young birds affected with cold should be examined for intestinal worms, and treated for same.

(2) Contagious Nasal Catarrh of Birds.

This is a contagious disease attacking principally the mucous membranes covering the eyes, the infra-orbital sinuses, the nostrils, and the trachea. The disease occurs preferably among young fowls, during damp, cold weather in autumn or spring. It is epizootic in character, and often causes the death of many birds. Its economic importance depends both (*a*) upon the fact that affected adult hens are unfavourably influenced as to their egg-laying capacity, and (*b*) upon the great mortality among young fowls.

The nature of the microbe which constitutes the virus of roup is not known, but it is contained in the secretions of the mucosa of the head. The contagion is generally brought into the poultry yard by infected birds purchased in markets, or by wild birds flying from one place to another. The saliva and the discharges from the nostrils and eyes carry the contagion, and soon contaminate the drinking water and food, so spreading the disease. Wind carrying the contagion, persons, animals, or birds that pass from one yard to another assist in the spread of the disease.

Symptoms.—The symptoms first seen are similar to those seen in simple catarrh, but they are more severe. The sick birds separate themselves from others, sit quietly with ruffled feathers and drooped wings. The nasal discharge is at first watery, but in two to three days it becomes thick, and has an offensive odour. The accumulation of mucus in the nostrils completely obstructs these passages, necessitating breathing through the mouth. Prolonged breathing through the mouth causes a horny, dried condition of the tip of the tongue. The inflammation, which begins in the nasal passages, soon extends to the eyes and to the infra-orbital sinuses, causing swelling of the eyes and face. The eyelids are swollen, and may be glued together by the accumulated secretion. In more severe cases the birds sit in a somnolent condition, unable to see and to eat ; they rapidly get weak, and many of them die within a week or ten days. Some of the affected birds recover, but others remain weak, and the disease assumes a chronic form. Such birds are dangerous to the flock, for they disseminate the contagion during the prolonged sickness.

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Treatment.—Sick birds should be removed from the flock and put in a warm, dry, well-ventilated house free from all draughts. The affected mucous membranes should be treated with antiseptic solution after washing well with warm saline solution containing 1 per cent. soda. The antiseptic solutions most suitable for such treatment are: boric acid, 5 per cent., hydrogen peroxide, potassium permanganate, 0.5 per cent.; copper sulphate, 2 per cent. Where the inflammation has extended to the eyes, these should first be washed well with warm saline solution, and two or three drops of a 15 per cent. solution of argyrol instilled into the eyes twice daily for several days. If there is swelling under the eye, it should be carefully opened with a sharp, clean knife, all the excretion removed, and the cavity washed with one of the above-mentioned antiseptic solutions. A pledget of cotton wool moistened with the solution may be kept in the cavity for one or two hours.

The food of sick birds should be soft and easily digested (see treatment of simple catarrh). Potassium permanganate should be added to the drinking water. The houses and their surroundings should be kept clean. If the disease is of a severe type, it is better to kill the affected birds: this avoids the retention of birds which may harbour the contagion and cause subsequent outbreaks.

(3) Nutritional Roup.

This condition attacks mostly young birds, and is similar to contagious roup, but is not infectious, and is due to the deficiency of vitamin A in the ration. Outbreaks are confined largely to the late summer and autumn months, those periods of the year in which it is difficult to raise green food. The disease occurs more frequently in fowls between the age of four and twelve months than in those younger or older. Young birds usually receive an adequate diet; older birds are apparently quite resistant.

Symptoms.—The characteristic symptoms of this disease are confined to the head, and involve the nasal passages, the mouth, pharynx, and œsophagus, and the eyes. There is nearly always present a watery discharge from the nostrils. Later this may collect in the infra-orbital sinuses, become transformed into a caseous mass, and cause swelling of the face. The face is now like one affected with contagious roup, and, in the absence of other symptoms described below, is of no diagnostic value. The lesions in the eyes consist of reddening of the conjunctiva and profuse watery secretion which soon becomes viscid, and may glue the eyelids together. These symptoms are followed by the formation of a tightly adherent white film over the *membrana nictitans.* The lesions in the mouth, pharynx and œsophagus consist of circular, white pustule-like caseous patches. These lesions commonly appear first in the pharynx and later on the tongue, on the floor of the mouth, and in the œsophagus. These pustules are associated with no other disease or condition in fowls. Marked emaciation and weakness occur in the later stages of the disease, although many fowls

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die suddenly while still in good flesh. In many cases there is a deposit of a chalk-like substance on the feathers below the vent. Post-mortem findings are: (1) Kidneys pale and marked by a network of fine white lines; (2) a deposit occasionally of urates on the heart, pericardium and liver.

Prevention.—It is difficult to treat and cure sick birds suffering from nutritional roup, but to prevent its occurrence one should supply the birds during the summer especially with green foods, such as green maize, alfalfa, carrots, yellow corn. Cold-liver oil given daily in 1 c.c. doses is an excellent prevention of this malady.

(4) Fowl-Pox—Oculo-Nasal Form.

Fowl-pox may be divided into the following three recognised clinical forms: (1) Wart-like nodules on the comb, wattles and skin of head. (2) Adherent, yellow, cheesy membranes in the mouth. (3) A watery or muco-purulent discharge from the eyes and nose. The prominent symptoms of the last-mentioned form are dullness, loss of appetite, sneezing, and an occasional cough. These symptoms are followed by a watery nasal discharge which gradually becomes purulent and obstructs the nasal passages. The eyes are swollen and inflamed, and discharge a viscid material which tends to stick the eyelids together. In many cases the head is swollen on one or both sides. Fowl-pox is a contagious disease, and is notifiable under Section 2 of the Diseases of Animals Ordinance, 1926.

Summary.

(1) *Simple Catarrh* (*Colds*) usually appears in sporadic cases, or it may also occur among a number of birds in a flock kept under favourable and hygienic conditions, but (*a*) it does not spread extensively, or, in other words, it is not infectious; (*b*) the nasal secretion remains usually thin and fluid; (*c*) the affected conjunctivæ and pharyngeal mucosa which in rare cases may exist remains moderate in degree.

(2) *Contagious Nasal Catarrh.*—(*a*) It is epizootic in character, and often causes the death of many birds; (*b*) a deposit of yellowish material may be found on the walls of the mouth and throat, but it is easily removed without injuring the underlying tissues.

(3) *Nutritional Roup.*—(*a*) The disease occurs more frequently in young birds in time of scarcity of green food; (*b*) the appearance on the mucous membrane of the mouth, pharynx and œsophagus of white pustule-like caseous patches; (*c*) the formation of a tightly adherent white film over the *membrana nictitans*, and the accumulation of a mass of white caseous material within the conjunctival sacs.

(4) *Oculo-Nasal Form of Fowl-Pox.*—Inflammation of the eyes and nose may be caused by agents other than the virus of fowl-pox, but when it is due to this virus, birds affected with characteristic comb and mouth lesions will be found simultaneously in the flock.

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POULTRY DISEASES IN CYPRUS.*

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Veterinary Service for Cyprus.

Fowl Cholera.—Three areas remained in quarantine on account of this disease at the end of 1929, and a further outbreak was dealt with in January, 1930. These areas were declared free in January and February, and no further cases were reported until September. Twenty-eight outbreaks were dealt with in the latter part of the year. Serum purchased from abroad appeared to give little protection in these cases, and much better results were obtained with a serum prepared at our laboratory from a local strain of *B. avisepticus*. Almost twelve thousand poultry were treated with serum by the field staff. Ten areas remained in quarantine at the close of the year.

Fowl-pox is of widespread occurrence in the Colony, and no administrative control has yet been attempted. The method of vaccination by pigeon-pox virus, as described by Doyle, has been tested on some three hundred birds, the vaccine being prepared from crusts obtained from pigeons artificially infected with crusts received from the Veterinary Laboratory, Weybridge. The results have been good except in one instance, in which twelve turkeys became infected naturally about fourteen days after vaccination. The vaccine will be used more extensively in 1931.

Avian Spirochætosis is very common in the island. Tick-control measures and the use of atoxyl intramuscularly for the treatment of affected birds usually succeed in checking the mortality.

Avian Tuberculosis, which had not previously been recorded in Cyprus, was found in a native hen purchased in Nicosia market. It is probable that the disease is not uncommon in the Colony, and there is some risk of its transmission to horses, cattle and pigs.

Fowl Cholera.—A strain of *B. avisepticus* isolated from a hen carcase from a local outbreak was used for the hyper-immunisation of two mares, one of which had produced almost 7,000 c.c. of serum before the end of the year. The serum did not always give protection against a subsequent inoculation of virulent culture, but this test was an extremely severe one. The use of the serum in the infected village areas was followed by results which were fairly satisfactory. In connection with work on this disease, Mr. Manley also reports as follows: "A forty-eight hour broth culture to which 2 per cent. formalin is added, and which is then incubated for twenty-four hours, is also being tested for immunising power. Balozet recommends that this vaccine be followed by an inoculation of virulent *B. bovisepticus* to create a permanent immunity, but since bovine hæmorrhagic septicæmia has not yet been recorded in Cyprus, it is thought that the use of this second inoculation is not advisable.

* From the 1930 Annual Report of the Chief Veterinary Officer.

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An experiment carried out with formalised spleen and liver, two inoculations being given with an interval of twenty-one days between, indicated that some immunity to subsequent inoculation with virus was produced. The tissues were obtained from a pigeon just dead from cholera. The quantity of tissues obtainable is so small that this method of vaccination would probably only be of practical value in special instances. However, it does suggest a type of vaccination which might be applied to bovines in those countries where hæmorrhagic septicæmia of cattle is of economic importance.

Fowl-pox.—The preparation of a vaccine against this disease, using pigeon-pox virus as recommended by Doyle, has been commenced, and its issue for more extensive use will be undertaken in 1931. A bacillus which morphologically resembles *S. gallinarum* has been isolated from an outbreak of disease in poultry. The identification has not been completed.

A gram-negative bacillus was isolated from an outbreak of disease in canaries which proved pathogenic when fed to fowls and on intraperitoneal inoculation of guinea-pigs. It is probably of the salmonella group. In three hens kept at the laboratory a disease was encountered simulating what is now known as "fowl paralysis."

Clinical Articles

Cerebro-Meningitis in Cattle.

By J. W. HALL MASHETER, M.R.C.V.S.
Newnham-on-Severn.

SINCE December 1st, 1931, I have had six cases of disease simulating the above, and I am recording these in the hope that similar cases may be reported. Every case I have had has died.

Case 1.—December 1st; died December 2nd, 1931. Case 2.—December 10th; died December 11th, 1931. Case 3.—December 11th; died December 12th, 1931. Case 4.—December 16th; died December 16th, 1931. Case 5.—January 14th; died January 14th, 1932. Case 6.—January 18th; died January 18th, 1932.

Case 1.—Roan cross-bred cow, six years old, in fair condition. She was turned out after milking on the evening of November 30th, and found down in the field first thing on the morning of December 1st; was put on a gate and drawn into the shed.

Symptoms.—Lying on her side with the body in a state of tonic spasm. Eyeballs fixed, pupils dilated; breathing accelerated; heart beats very strong and

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quick; sweating; temperature 103° F.; passed both fæces and urine. She had quiet periods, but the least movement or sound brought on severe convulsions. Died 10 a.m. December 2nd.

Case 2.—Young shorthorn cow in very good condition. She was turned out after milking on the evening of December 9th, and was brought in again at milking time on the morning of December 10th. She was noticed to walk in a blind way, with inco-ordination of movement of all four legs; she was milked, and gave her usual quantity of milk, and then "had a fit." The symptoms on my arrival were as in Case No. 1: Temperature 104.2° F. She died at 5 a.m. on December 11th.

Case 3.—Young shorthorn cow in very good condition. She was turned out after milking on December 10th, apparently quite healthy, and on being brought in at milking time on the morning of December 11th, was noticed to be restless, and continually lowing, as it might be to call her calf (she had been weaned from it for over three weeks). I saw her on the night of December 11th. She was very restless, walking round the box with head stretched out, and still lowing. Temperature, 104° F.; sweating, and seemed to be in slight abdominal pain; abdomen tucked up, and I noticed, too, that she kept curling her tail over the hind quarters. Could not rest, was continually lying down and rising again. I left a sedative draught. I saw her again on the morning of December 12th. She was down, and showing the marked symptoms of Cases 1 and 2. She died at 10.30 a.m.

Case 4.—A black Welsh cow, three years old. She was turned out on the morning of December 16th, and seemed to be all right. At 3.30 p.m., when being brought in for the evening milking, was seen to have some difficulty in walking, but eventually was got into the shed, when she laid down. I saw her about 4 p.m., and had no difficulty, after my previous experience of these cases, in diagnosing the condition, and giving an unfavourable prognosis.

Symptoms.—Grinding her teeth and moaning; sweating; hurried breathing; head extended in a straight line; eyes fixed; easily excited; and unable to rise. Temperature 103° F. She died about 11 p.m.

Case 5.—Red shorthorn cow, eight years old, in good condition. I saw this cow about 8.30 a.m. on January 14th, 1932. She was recumbent, grinding her teeth, and sweating; head extended in a straight line with the neck; eyes staring, pupils dilated, and was quite blind; moaning; temperature, 104.1° F. She had several convulsions during the day, and died at 6 p.m.

Case 6.—Red and white shorthorn cow in good condition, four years old. She was milked, and seemed all right on the night of January 17th, 1932. At 6 a.m. on January 18th, the owner found her "in a fit, which she never came out of." She was dead before my arrival at 8.30 a.m. I found the body lying on the side with the legs stretched out at right angles. Rigor mortis was very marked, and the body bathed in perspiration.

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I post-mortemed three of these cases, removing the brain, but beyond a reddening of the meninges they showed very little macroscopically. In Case 6 there was a considerable amount of cerebral fluid, cloudy, and of a reddish colour. Owing no doubt to bad post-morteming on my part, I did not notice the fluid of the others (the head was detached from the body before removing the brain, and no doubt the fluid drained off). The lungs in all cases were engorged with blood, and the heart also " filled to bursting," probably due to a gradual paralysis. Macroscopically, behind the diaphragm, all the organs appeared to be healthy.

Treatment was of no avail. The first two or three cases I took to be " tetany," and tried injections of calcium gluconate, and injecting the udder with oxygen. In other cases I tried sedatives, but nothing had the least effect. It will be noticed that all the cases were cows in milk, and that most of them were young and in good condition. No two cases were found on one farm.

Case 2 was seven miles from Case 1, with the River Severn in between. Case 3 was seven miles from Case 2, and about three miles from Case 1. Case 4 was three miles from Case 3. Case 5 was thirteen miles from Cases 1, 2 and 4. Case 6 was eight miles from Case 5. Only two cases, Nos. 2 and 3, occurred on low-lying land, the other four being at an altitude of from 150 to 600 feet above sea level.

I base my diagnosis of these cases on the symptoms of " Other Enzootic Forms of Meningo-Encephalomyelitis," as described by Hutyra and Marek in their special pathology and therapeutics, Vol. III, third edition: " Prodromal symptoms are noted in some cases, *viz.*, dullness, listlessness, compulsory movements. Not infrequently the disease commences very suddenly with severe manifestations, particularly in cases associated with the formation of a copious purulent exudate. In correspondence with the frequent intensity of the meningitic exudate, the signs of excitement and depression are severe, and increase rapidly, and the manifestations of irritation, induced by irritation or injury of the cerebral and spinal nerve roots, appear very early, and are marked. This is true particularly for the stiffness of the neck and of the muscles of the back, which causes the back and neck to be held straight (orthotonus) or to be bent backwards (opisthotonus); in other cases the neck and trunk are bent sideways (pleurothotonus); it is also true for the disturbances in deglutition, which are produced by cramps in the muscles of the gullet. At times the lower abdomen is seen to be hunched up on account of prolonged cramps in the abdominal muscles; the stiffness of the muscles of the extremities cause the feet to be spread apart. Trismus may make its appearance, as well as oblique position of the eyes, nystagmus, unequal dilation of the pupils. Transitory local muscular cramps in the muscles of head, neck, trunk and extremities have been noted before the appearance of the permanent muscular rigidity, and also in cases not associated with stiffness of the muscles. A frequent symptom is hyperæsthesia with increased reflex irritability (the animals are easily frightened, and convulsions occur on slight external stimuli)."

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Reviews

Der Aseptic in die Veterinarchirurgie (Asepticism in Veterinary Surgery). 1st edition. By Dr. Jordanoff, Professor of Surgery and Canine Diseases, and Director of the Animal Clinic in Erfurt; and District Veterinary Surgeon in Bulgaria. Possneck: Gerold. 1931. Pp. 169; 102 figures in the text.

THIS book, written in German, and dedicated to several professors of the Hanover School, deals with the whole range of producing asepticism in veterinary surgery. It points out the ideals that should be striven after in a teaching school where money and assistance are both plentiful. In this connection it will be more useful and valuable to veterinary colleges and faculties than to the majority of veterinary practitioners. The illustrations are numerous and well produced, but whether even in the most elect circles veterinary surgeons always operate with sterile mantles, headpieces, and indiarubber gloves, is at least doubtful. In a hospital operating room dealing with humans no doubt every precaution to secure asepticism is carried out, but with animals the same degree of asepticism cannot be carried out or maintained. As a general survey of the whole field of aseptic surgical technique, the book is to be recommended. Here and there the practitioner may glean some tips that may help him on his way, but it would probably take him a lifetime to carry out all that is recommended. There are twelve chapters in the book, including: antiseptics and asepsis, modern veterinary surgical procedure, preparation of the patient, the field of operation and hands of the surgeon and assistants, and the instruments before and during the operation. Narcosis and anæsthesia are discussed in fifty-three pages. Rectal avertin narcosis has three pages. In the human subject, the observers he quotes say: "the older the better." The most difficult dosing was in patients between fifteen and thirty-five years of age. The author has no experience of avertin himself, but it seems from the reading that German experiences with this drug in dogs and cats are few, and the conclusions not reliable.

The Cause of Cancer. By W. E. GYE, M.D.; and W. J. PURDY, M.B. London: Cassell & Co. 1931. Pp. xiv + 515. Price 30*s*.

THE authors deserve the highest praise for their arduous contribution in the study of cancer. Their numerous experiments, observations, and deductions, although naturally limited to one aspect of the disease, are most valuable, and should be perused by all investigators in this field of work. In brief, their work has more than fully justified their original conception that one of the primary factors in sarcomatous and allied conditions in the domestic hen is a filtrable virus. A virus has never been demonstrated in mammalian neoplasms, but Gye and Purdy suggest that this may be due to imperfect technique rather than to the absence of a virus. The work is written in remarkably simple and clear language, quite understandable by the average student and is divided into four main sections: (1) Known facts concerning tumours of man and mammals; (2) known facts concerning tumours of fowls; (3) the authors' experiments; (4) review. The third section comprises four-fifths of the whole volume, testifying to the completeness of the work.

W. P. B.

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News

A RACING Lads' Boxing Tournament was held on February 9th, at the Royal Albert Hall, on behalf of the Re-building and Endowment Fund of the Royal Veterinary College, and the Heritage Craft Schools, Chailey. The affair was arranged as a series of contests between the stable lads of Newmarket and those of the South of England, and the various trainers entered into the spirit of the game as eagerly as the boys themselves. The prizes included some very valuable silver cups; and the Challenge Trophy (worth 200 guineas) was presented by Sir Malcolm McAlpine. This finally fell into the hands of the lads of the South, as they outclassed their opponents from Newmarket in a most remarkable manner, winning every contest.

His Royal Highness the Prince of Wales, whose willingness to assist every deserving cause is so well known, very kindly attended in person and presented the prizes, much to the delight of the young boxers, whose weights ranged from 8 stone down to as low as 4. It is proposed to make the affair an annual one.

SINCE Dr. Leclainche, of the Ministry of Agriculture, and Professor Nicolas created a station for the study of the hygiene and pathology of poultry at the Alfort Veterinary School, 10,500 consultations on their diseases had taken place from November 15th, 1928, to June 15th, 1929.

THE NATIONAL GREYHOUND RACING SOCIETY.

President.—The Lord Askwith, K.C.B., K.C. *Vice-Presidents.*—Sir William Gentle, J.P.; Major-General The Lord Loch, C.B., C.M.G., D.S.O.

Council.—Admiral Sir Sydney Fremantle, G.C.B., M.V.O.; Brig.-General A. C. Critchley, C.M.G., D.S.O.; W. H. McGrath, Esq.; Lieut.-Colonel H. A. Browne; H. Garland Wells, Esq.; Capt. M. W. A. P. Graham, M.C.; W. J. Cearns, Esq.; James B. Fraser, Esq.; Lieut.-Colonel E. L. Mears, D.S.O.; Lieut.-Colonel G. R. Powell, O.B.E., J.P.; Sir Louis Dane, G.C.I.E., C.S.I.; F. S. Gentle, Esq. *Secretary.*—Major C. E. Lucas Phillips, M.C.

Stewards of the National Greyhound Racing Club.—Henry Sawtell, Esq.; The Lord Lawrence, D.L.; Captain C. R. Jorgenson; Brigadier-General The Hon. Alick Russell, C.M.G., D.S.O.; Professor F. T. G. Hobday, C.M.G., F.R.C.V.S.; Captain B. F. R. Fairfax-Lucy. *Senior Stipendiary Steward.*—Lieut.-Colonel R. Cockburn, O.B.E. *Secretary.*—Lieut.-Colonel Romer Baggallay, D.S.O., M.C.

Official statistics of Greyhound Racecourses affiliated to the National Greyhound Racing Society and licensed by the National Greyhound Racing Club: *Total Attendances*: 1927, 5,656,686; 1928, 13,695,275; 1929, 15,855,162; 1930, 17,119,120; 1931, 17,906,917.

Total Prize Money paid to owners of greyhounds (figures for four years only), £1,075,000. Total Entertainments Tax (for five years), £733,205. Total payments to Charities (for five years), £53,000 (approximately).

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Notices

"SALCA," which is described by the makers, the Associated London Flour Millers, Ltd., as "iodised minerals," has been on the market for a number of years, during which time it has proved an efficient supplement to the diet of dogs as it supplies the mineral elements which are frequently lacking, and which are so necessary for the promotion of health, growth and energy. Reports state that there is considerable improvement in health to be derived by the use of this preparation. The dosage is as follows:

	Teaspoonfuls per head per day.
Group I.—Poms, Pekinese, Small Terriers, etc.	½
Group II.—Terriers, Sealyhams, etc.	1
Group III.—Greyhounds, Alsatians, etc.	1½
Group IV.—Mastiffs, Gt. Danes, etc. (Foxes as Group II.) ...	2

Personal

MR. T. EATON JONES, F.R.C.V.S., the popular Chief Veterinary Official of the City of Liverpool, is due to be placed on the retired list next month, after thirty-six years' service; during thirty-one of which he has occupied his present post.

Mr. Eaton Jones, who succeeded Mr. Reynolds, who was also a very popular and efficient organiser, has been the reliable guide of the Council officials in many of their plans for the benefit of the City, and the ratepayers have on more than one occasion had reason to be grateful to his advice for efficient economies in connection with the transport services of the Corporation. His geniality towards his veterinary brethren is well known, and his colleagues in the profession will wish him long-continued good health to enjoy the term of well-earned rest which lies in front of him.

MAJOR H. H. BRASSEY-EDWARDS, the Chief Veterinary Officer of Kenya Colony, has now been appointed Deputy Director of Agriculture and Animal Industry.

CAPT. T. M. DOYLE, F.R.C.V.S., D.V.S.M., of the Ministry of Agriculture, delivered two very interesting lectures on "Diseases of Birds due to Filtrable Viruses," on the 19th and 26th of February, in the large lecture theatre of the Royal Veterinary College, under the auspices of the University of London. The Chair was taken on the first occasion by Sir Edward Brown, LL.D., F.L.S., and on the 26th by Major-Gen. Sir John Moore, K.C.M.G., C.B., F.R.C.V.S. The lectures were well attended.

Publishers' Notices

All communications should be addressed to 7 & 8, Henrietta Street, Covent Garden, London, W.C. 2. Telephone: Temple Bar 4646. Telegrams: "Baillière, Phone, London."

Letters for the JOURNAL, literary contributions, reports, notices, books for review, exchanges, new instruments or materials, and all matter for publication (except advertisements) should be addressed to the Editor.

Copy of advertisements should be in the hands of the publishers—Baillière, Tindall and Cox—not later than the 25th of the month, or if proof is required, not later than the 23rd.

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